

Hypogeous fungi in the diet of the red-legged pademelon *Thylogale stigmatica* from a rainforest-open forest interface in northeastern Australia

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ABSTRACT

The diet of the Red-legged Pademelon *Thylogale stigmatica* has previously been described as comprising a range of dicotyledonous and monocotyledonous plants, rainforest fruits, seeds, and some fungi. We collected *T. stigmatica* faecal samples from a rainforest-open forest ecotone in northeastern Australia and analysed them for the presence of fungal spores. Of the 20 samples collected, 12 contained spores of several types of hypogeous fungi, with the number of spore types per sample ranging from 1-7, with a mean of 5. Twenty fungal spore types were recognised in total; seven of these could be attributed to hypogeous ('truffle-like') ectomycorrhizal genera, and most others had spore morphologies suggestive of a hypogeous habit. This is the first report of consumption of ectomycorrhizal hypogeous fungi by the genus *Thylogale*, and as such, adds important new information on the role these forest-dwelling wallabies might play in dispersing hypogeous fungi across the dynamic interface between rainforest and open forest in eastern Australia.

Key words: mycophagy, spore dispersal, ectomycorrhizal hypogeous fungi, truffle, ecosystem dynamics, rainforest, pademelon, marsupial.

Introduction

Hypogeous ectomycorrhizal fungi form important symbiotic associations with woody plants of forested ecosystems (Smith and Read, 1997), and their mature fruit-bodies emit strong odours that attract mycophagous mammals (Donaldson and Stoddart, 1994) that ingest the fungi and ultimately disperse their spores (Claridge *et al.* 1996). Spore-laden animal scats concentrate spores at points in the soil where animals have defecated, thereby providing a more effective inoculum than a few dispersed spores (Trappe and Claridge 2005).

Hypogeous ectomycorrhizal fungi are richly diverse in Australia, with perhaps more than 2000 species, although many of these remain undescribed (Bougher and Lebel 2001). Eastern Queensland alone probably has upwards of 300 species, as many or more than is known from all of Europe. This extraordinary diversity attests to the effectiveness of the selection pressure towards evolution of the hypogeous habit in the region, as well as the importance of these fungi in its forest ecosystems. Previous work in Australia has mostly concentrated on potoroid marsupials, bandicoots, and small rodents as dispersal vectors for hypogeous fungi (see Claridge 2002). However, because hypogeous fungi rely primarily on mycophagy for their spore dispersal (Trappe and Claridge 2005), we expect there to be a greater diversity of mammals in eastern Australia that consume and disperse fungi than those currently documented.

The Red-legged Pademelon *Thylogale stigmatica* (Figure 1) is a potential candidate for dispersal of hypogeous fungi. This small macropodid marsupial inhabits tropical and



Figure 1. A red-legged pademelon *Thylogale stigmatica* in rainforest at Lake Eacham, northeastern Queensland. Photo: Peter Jarman.

sub-tropical rainforest and other closed forest habitats in eastern Australia and New Guinea (Johnson and Vernes 1995) and, in northeastern Queensland, seems to be most common where rainforest adjoins pasture or grassy woodlands. It browses leaves, fruit and seeds of rainforest plants by day, venturing to the forest edge at night to graze primarily on grasses (Vernes 1995; Vernes *et al.* 1995). Redenbach (1982) reported small amounts (1-2%) of unidentified fungi in the diets of *T. stigmatica* and the closely related Red-necked Pademelon *T. thetis*, and from a collection of five scat samples in northeastern Queensland, Reddell *et al.* (1997) found a few spores of an arbuscular mycorrhizal fungi in one of them. Other researchers have either not detected or disregarded

fungi in pademelon diets. Because pademelons are ecotonal mammals, they may be important dispersers of spores between rainforest and open forest ecosystems. Such processes can prepare a potential soil spore bank for fluxes of plant host species from one community to another (Barr *et al.* 1999, Kjøller and Bruns 2003) thereby enabling mycorrhiza formation and hence establishment of invading species even as they enable fungi to initiate and develop new colonies.

Our paper presents data on consumption of ectomycorrhizal hypogeous fungi by *T. stigmatica*. One cannot quantify the amount of fungi in relation to other foods that browsing animals consume by examination of faeces, because different foodstuffs are differentially digested. However, the relative abundance and diversity of spores offer clues to the extent of mycophagy in the diet of an animal. Accordingly, our data provide a basis for hypothesizing about the importance of this phenomenon to *T. stigmatica* nutrition and to the ecology of the dynamic interface between rainforest and open forest in northeastern Australia.

Methods

The study area was located primarily on the western side of the Mount Windsor Tableland ($16^{\circ}13'32"S$, $145^{\circ}02'36"E$) in northeastern Queensland, Australia, part of the Australian Wet Tropics World Heritage Area. Here, 18 *T. stigmatica* faecal samples were collected at an abrupt natural ecotone between tropical rainforest and open wet sclerophyll *Eucalyptus* woodland. Each sample represented a separate, recent defecation by a pademelon, and although the collection was opportunistic, we chose samples as far from one another as possible within an area *ca.* 50 x 50 m. Previous work on the nocturnal range of *T. stigmatica* in north Queensland (Vernes *et al.* 1995) suggests that these samples would have come from more than one or two individuals. Additionally, we collected two separate *T. stigmatica* faecal samples from rainforest habitat at Lake Eacham on the Atherton Tableland ($17^{\circ}16'16"S$, $145^{\circ}38'02"E$), also in northeastern Queensland. The rainforest at Lake Eacham is surrounded by cleared pasture, but there are appropriate plant hosts for ectomycorrhizal fungi (e.g. *Eucalyptus* spp.) as tall emergent trees within the forest near where the samples were obtained.

Faecal samples were briefly softened in 5% KOH, macerated, and then rinsed with distilled water through a 125 x 125 μm mesh. Two to three drops of the mixture that passed through the mesh were mounted on a glass slide with glycerine jelly and topped with a glass cover slip. Slides were systematically scanned at x400 under a light microscope; x1000 magnification was used to define morphologic characters (size, shape, ornamentation, wall thickness, and symmetry) of each spore type encountered, and to identify them to the lowest taxonomic level possible. The six samples with the greatest diversity and apparent density of spores were also examined with a scanning electronic microscope (JEOL JSM-5600 SEM operating at 10 kV and 8.48 mm

working distance) to obtain representative photographs of as many of the spores as possible.

Hypogeous fungi have been collected in both rain forests and open forests in tropical Queensland since 1988 by N. Malajczuk, J. Trappe, M. Castellano, N. Bouger, P. Reddell and colleagues during eight expeditions. Two of these included brief visits to the Mount Windsor Tableland. Along with Queensland collections by K. Vernes, nearly 1,500 reference collections are available in the National Herbarium of Victoria, Melbourne; Western Australian Herbarium, Perth; and temporarily in the Mycological Herbarium of Oregon State University, Corvallis, as a framework for identifying spores of hypogeous taxa in animal faecal samples from the region. These herbarium collections were identified to genus and, when possible, to species by J. M. Trappe, M. A. Castellano and T. Lebel and were available for our study.

Results and Discussion

Twelve of the 20 *T. stigmatica* samples we examined contained fungal spores, with some containing as many as seven spore types (mean taxa per sample = 5). No two samples that contained fungi were identical in their composition of taxa, suggesting that the faecal collections represented the diets either of several individual animals or of a few animals over several nights of foraging. Twenty fungal taxa were identified in these samples (Table 1). The grouping of taxa reflects the level to which we could identify them by microscopic spore characteristics alone. Eight could be identified to genus (including one being identified to species), whereas others were grouped in families with genera that cannot be distinguished by spore characteristics alone. These were the Russulaceae, including the genera *Cystangium*, *Gymnomycetes*, *Macowanites* and *Zelleromyces*, and the Tuberaceae, including the genera *Labyrinthomyces*, *Reddellomyces* and *Dingleya*. All seven genera occur in northeastern Queensland. Several spore types could not be equated with any described genus and are listed as 'Unknown'. All but four of the types seen are probably hypogeous, mycorrhiza-forming groups (Table 1). Figure 2 shows photos of some of the spore types we obtained using scanning electron microscopy.

Past collecting of hypogeous fungi in Queensland has produced representatives of about 42 genera distributed among 16 families, 10 in the Basidiomycota, 4 in the Ascomycota, and one each in the Zygomycota and Glomeromycota (Trappe and Castellano, unpublished data). The two, brief fungal forays held in the Mount Windsor Tableland in the past yielded 12 species representing eight families. Only five taxonomic groups of the Mount Windsor collections overlapped with the 14 groups found in the pademelon scats (Table 1).

From examination of stomach contents, Redenbach (1982) estimated the diet of both *T. stigmatica* and *T. thetis* in northeastern New South Wales to be comprised of about 1-2% fungi on average, with a maximum of 8% of material in stomachs being fungus. Other studies have not searched for fungi in pademelon diets (e.g.

Table 1. Fungal diversity in the diet of red-legged pademelons *Thylogale stigmatica* determined from faeces collected on The Windsor Tableland (W) and at Lake Eacham (E), in northeastern Queensland.

Fungal taxa	Growth habit	Faecal sample location	Number of samples in which present
<i>Chamoniaxia</i>	hypogeous	W	3
<i>Cribbea</i>	hypogeous	W	1
<i>Cribbea</i>	hypogeous	E	1
<i>Gautieria</i>	hypogeous	W	7
<i>Gymnohydnotrya</i>	hypogeous	W	2
<i>Hydnoplicata</i>	hypogeous	W	1
<i>Hysterangium</i>	hypogeous	W	7 ¹
<i>Sphaerosoma</i>	hypogeous	W	1
<i>Stephanospora flava</i>	hypogeous	W	1 ¹
Russulaceae 1 (Possibly <i>Gymnomycetes</i>)	hypogeous	W	6 ¹
Russulaceae 2	hypogeous	W	8 ¹
Russulaceae 3	hypogeous	W	1 ¹
Tuberaceae	hypogeous	W	3 ¹
Cortinariaceae (possibly <i>Thaxterogaster</i>)	hypogeous	W	1
Cortinariaceae (possibly <i>Thaxterogaster</i>)	hypogeous	E	1
Unknown 1	hypogeous ²	W	1
Unknown 2	hypogeous ²	W	1
Unknown 3	hypogeous ²	W	3
Unknown 4	hypogeous ²	W	1
Unknown 5 (Possibly <i>Octaviania</i>)	hypogeous	W	1
Unknown 5 (Possibly <i>Octaviania</i>)	hypogeous	E	1
Unknown 6 ('mushroom')	epigaeous	W	2
Unknown 6 ('mushroom')	epigaeous	E	2
Unknown 7 ('mushroom')	epigaeous	W	1
<i>Microthecium beatonii</i>	parasitic on truffles	W	1

¹Species, genera or families also collected in two forays for hypogeous fungi in the Mount Windsor Tableland.

²Although not identified to family or genus, the symmetrical shape of the spores indicates statismospores, i.e., lacking a forcible spore discharge mechanism as is typical of the hypogeous habit.

Vernes 1995; Sprent and McArthur 2002), and it is likely that fungus is a minor and probably overlooked component of pademelon diets. Fungi thus might seem to be insignificant in terms of their dietary volume. However, as accumulators of minerals they may be akin to salt licks, and are sources of many vitamins and amino acids (Claridge and Trappe 2005). Moreover, the immense numbers of spores present in even a small amount of ingested fungi and the diversity of taxa present in the samples we analysed have potentially important ecological ramifications. Previous work on a range of mammals has shown that spores of hypogeous fungi remain effective mycorrhizal inoculum after passage through the digestive tract (e.g. Claridge *et al.* 1992; Reddell *et al.* 1997; Claridge *et al.* 2001, Caldwell *et al.* 2005), so pademelons probably serve as dispersal vectors for a range of fungi that occur near rainforest edges.

Our work is the first to report consumption of hypogeous ectomycorrhizal fungus by the genus *Thylogale*, and it is one of only a few that report mycophagy by macropodid

marsupials (Christensen 1980; Claridge and May 1994; Reddell *et al.* 1997; Claridge 2002; Claridge *et al.* 2001). Claridge *et al.* (1996) warn that because fungal spores are typically minute, they can be overlooked in diets. Our data indicate that this has probably been the case for pademelons and suggests that the importance of other macropodids as dispersers of hypogeous fungi is likely to emerge when assessment of fungal spores in diets is included in analytical protocols.

On the Atherton Tableland in north-eastern Queensland, Vernes *et al.* (1995) showed that *T. stigmatica* is a species that associates with the interface between rainforest and adjacent open habitats, sheltering and browsing within rainforest by day and coming to the forest edge each night to graze on the grasses that abound there. Although the forest edge in Vernes' study abutted human-cleared pastures, pademelons have probably always associated with natural rainforest edges that offered the cover of rainforest juxtaposed with the abundant forage characteristic of adjacent *Eucalyptus* woodland. These natural edges, that still characterise

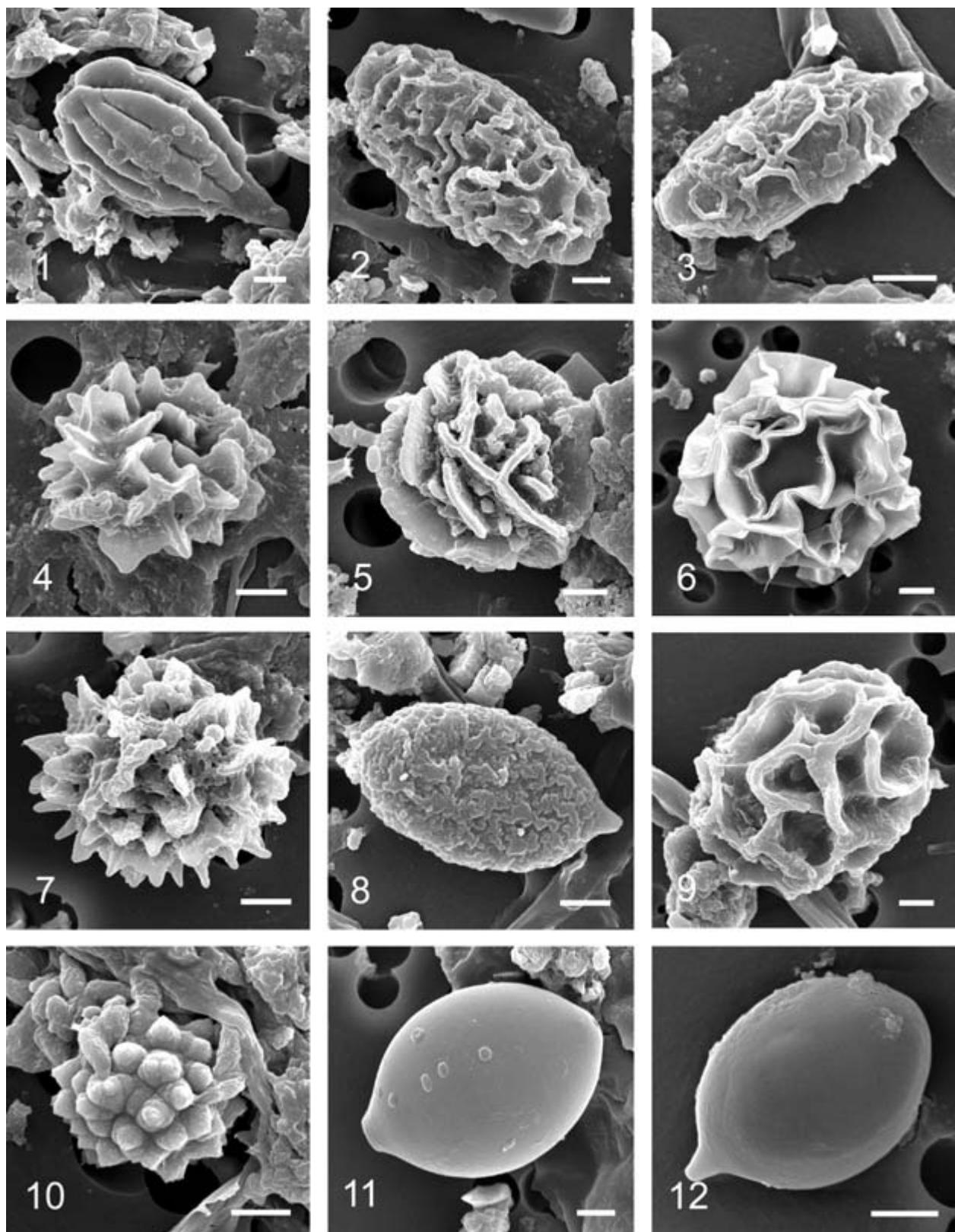


Figure 2. Examples of fungal spores in the diets of red-legged pademelons (*Thylogale stigmatica*) from northeastern Queensland. Spores were photographed with a scanning electron microscope from samples prepared from *T. stigmatica* faecal pellets. Key to spores: 1. *Gautieria*; 2. *Gymnohydnomyia*; 3. *Hysterangium*; 4. *Stephanospora flava*; 5. *Russulaceae* 1 (Possibly *Gymnomyces*); 6. *Russulaceae* 2; 7. *Russulaceae* 3; 8. *Cortinariaceae* (possibly *Thaxterogaster*); 9. Unknown 1; 10. Unknown 5 (Possibly *Octaviania*); 11. Unknown 6; 12. Unknown 7. All scale bars represent 2 μ m.

the transition from rainforest to open forest along much of the boundary of the Wet Tropics, are typically abrupt ecotones maintained in part by fire (Harrington and Sanderson 1994). The location of the rainforest-open forest ecotone depends on recent fire regimes (Unwin 1989, Ash 1988, Harrington and Sanderson 1994). Fires that periodically sweep into the region from the dry sclerophyll forests to the west are at times so intense that, under optimal burning conditions, they burn into the relatively less flammable rainforest, killing rainforest trees and clearing the way for the advance of the *Eucalyptus*-dominated open forest. At other times, runs of wetter years allow the rainforest to encroach upon the open forest (Unwin 1989). As time since fire increases, establishment of rainforest invaders in open forest reduce the habitat susceptibility to fire, and these habitat types may then trend toward rainforest (Webb and Tracey 1981).

Because rainforest trees mostly form mycorrhizae with vesicular-arbuscular fungi, and *Eucalyptus* and other open-forest trees are associated more with ectomycorrhizal fungi (Brundrett 1991), the dynamic rainforest-open forest edge also requires dispersal of the appropriate fungi back and forth across the ecotone if trees are to establish and one or the other forest type is to dominate the transition zone. *T. stigmatica* may well play an important role along with other animals such as bettongs (*Bettongia*), bandicoots (*Perameles*) and small rodents (e.g. Tory *et al.* 1997; Vernes *et al.* 2001) in the dispersal of both vesicular-arbuscular and ectomycorrhizal fungi, because they forage in both rainforest and open forest habitats (Vernes *et al.* 1995; Vernes 1995) and are shown by our data to consume a diversity of hypogeous species. Even though our samples were collected from a small area during one time period, the richness of fungi within them suggests that pademelons can be significant dispersers of fungi either side of the ecotone.

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